Principal Investigator: Dr. Monica Perez PT, PhD

Protocol Title: Maximizing Spike Timing-Dependent Plasticity After Spinal Cord Injury

A. Specific aims and hypotheses

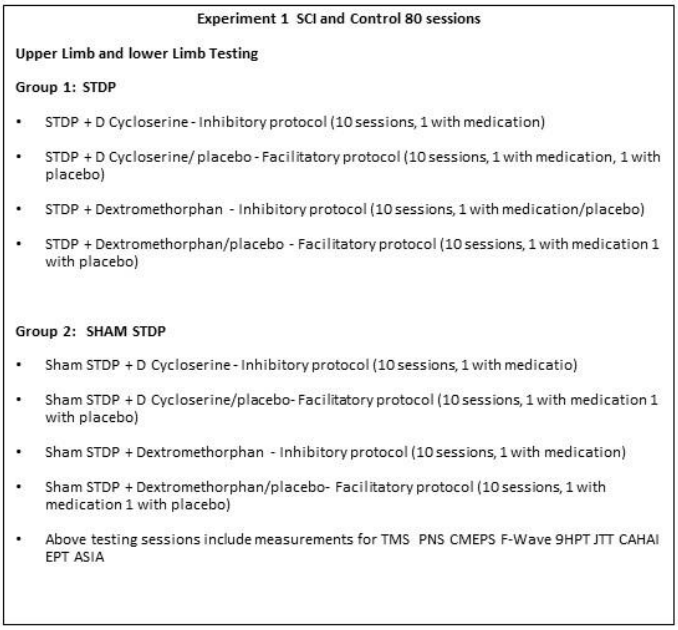
Aim 1. Maximize Spike Timing-Dependent Plasticity (STDP)-mediated motor function recovery in humans with incomplete SCI. To accomplish this aim we propose to complete 2 main experiments. First, we will combine STDP at the most optimal duration and frequency with the most effective receptor agonist D-Cycloserine, to maximize STDP aftereffects. Second, we will combine training with STDP-induced plasticity to further maximize voluntary output. We will test the hypothesis that maximizing the aftereffects of spike-timing dependent plasticity will further enhance hand and leg motor function in humans with incomplete spinal cord injury.

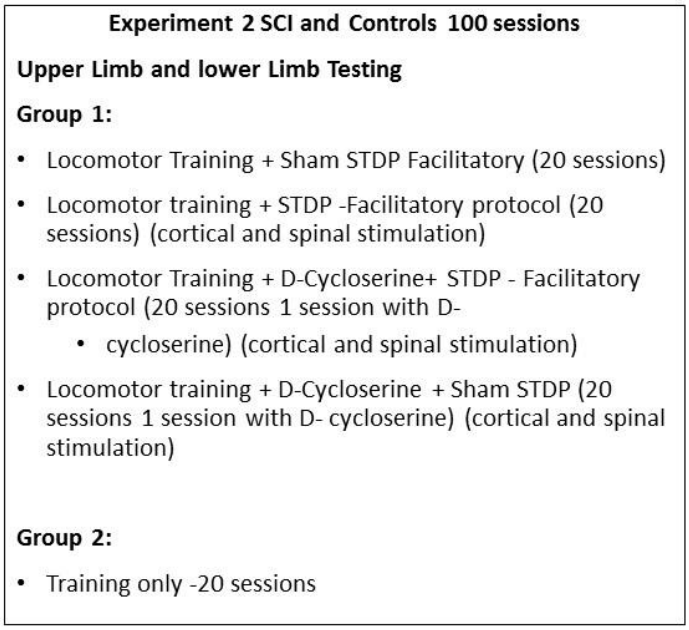
B. Background and Significance

Prevalence of SCI in VA population: More than 40,000 veterans have a spinal cord injury (SCI) causing functional impairments impacting their independence and social participation. Currently, about 26,000 veterans with SCI are cared for in the VA health system (Fact Sheet: VA and SCI, 2009).
Problem: Most SCIs are contusions and ~50% occur at the cervical level causing anatomically incomplete damage and bilaterally functional deficits to lower- and upper-limbs (Kakulas, 1999). Arm and hand function deficits limit daily-life activities, such as reaching and grasping, eating, drinking, and writing, which decreases the quality of life (Snoek et al., 2004; Herrmann et al., 2011). Regaining arm and hand function is considered the highest priority for improving the quality of life of quadriplegics (Anderson, 2004). Beneficial effects of upper-limb training after cervical SCI have been shown, but the functional improvements are limited (Spoorenet al., 2009). There is an unmet need for novel mechanism-driven therapeutic interventions for enhancing upper-limb motor function after cervical SCI.
[Contribution to the VHA: Within the VHA system, about 50% of the veterans with SCI has limited use of their arms and hands impeding their daily life activities and thus requiring continuous added care. For this group of veterans, effective approaches to enhance upper-limb function would be an important vertical step in increasing their: 1) daily independence and social participation, 2) chances to return to the work floor, 3) overall health and family partaking. For the VHA system, developing such an approach would significantly reduce the need for its involvement in the care of our veterans with SCI. Importantly; our results might impact the effectiveness of strategies to improve upper-limb function of 260,000 other veterans that currently live with a traumatic brain injury and 6,000 veterans that suffer a stroke per year.]

C. RESEARCH DESIGN AND METHODS

Aim 1. Maximize STDP-mediated motor function recovery in humans with incomplete SCI. To accomplish this aim we propose to complete 2 main experiments. First, we will combine STDP at the most optimal duration and frequency with the most effective receptor agonist (identified in Aim 1) to maximize STDP aftereffects. Second, we will combine training with STDP-induced plasticity to further maximize voluntary output. We will test the hypothesis that maximizing the aftereffects of spike-timing dependent plasticity will further enhance hand and leg motor function in humans with incomplete spinal cord injury.
Participants. We will test 250 individuals with incomplete SCI and 250 healthy controls. These numbers include an estimated subject attrition rate of 25%. Subjects will be recruited through research registries at the Miami VA and the University of Miami and The Miami Project to Cure Paralysis. In order to have our study group as homogeneous as possible, we will only include: (1) Males and females between 18-85 yrs, (2) SCI, (3) Spinal cord injury at L5 or above, (4) Intact or impaired but not absent innervations in dermatome C6 using the American Spinal Injury Association sensory scores, and (5) Ability to reach/grasp a small object located ~8 cm forward, above, and laterally without leaning forward with the trunk. See additional criteria in inclusion and exclusion section.





EXPERIMENT 1(Crossover design)**:** Approximately 160 sessions over the course of the study

Patients and healthy controls will be randomly assigned to 2 groups: (1)
STDP, and (2) sham STDP. STDP will be given at stimulation parameters and combined with the receptor agonist identified in Aim 1. Based on our preliminary data we expect that D-cycloserine will be the most effective agonist. In the STDP group, sessions will be completed separated by at least 3 days (facilitatory STDP+D-cycloserine, inhibitory STDP+D-cycloserine). In the sham STDP group,1 session will be completed with a placebo drug (sham STDP+placebo). Within experiment 1, patients and controls will participate in both groups.

SCI and Controls - Upper Limb and lower Limb Testing

Group 1: STDP
STDP + D Cycloserine - Inhibitory protocol (10 sessions, 1 with medication)
STDP + D Cycloserine/ placebo - Facilitatory protocol (10 sessions, 1 with medication, 1 with placebo)
STDP + Dextromethorphan - Inhibitory protocol (10 sessions, 1 with medication/placebo)
STDP + Dextromethorphan/placebo - Facilitatory protocol (10 sessions, 1 with medication 1 with placebo)

Group 2: SHAM STDP
Sham STDP + D Cycloserine - Inhibitory protocol (10 sessions, 1 with medication)
Sham STDP + D Cycloserine/placebo- Facilitatory protocol (10 sessions, 1 with medication 1 with placebo)
Sham STDP + Dextromethorphan - Inhibitory protocol (10 sessions, 1 with medication)
Sham STDP + Dextromethorphan/placebo- Facilitatory protocol (10 sessions, 1 with medication 1 with placebo)

For SCI subjects, ASIA and Electrical Perceptual Threshold (EPT) exams will be performed to assess the subjects sensory and motor function level.

STDP protocol: We will pair presynaptic volleys (produced by TMS) and postsynaptic volleys (produced by peripheral nerve stimulation) to arrive at the spinal cord at the most effective frequency and duration. [Based on our pilot data we expect these to be 20 Hz for 20 min. TMS intensity will be adjusted to produce MEPs of threshold size (0.05 mV) in the FDI.] Peripheral nerve stimulation over the ulnar nerve will evoke a response 5% of M-max in the FDI muscle. Arrival time of impulses at the presynaptic terminal will be based on central conduction time: MEP latency-[(F-wavelatency+M-maxlatency-1/2)]. Arrival time of impulses at the postsynaptic terminal will be based on peripheral conduction time: [(F-wavelatency+M-maxlatency-1/2)]. We will use ISIs with peripheral nerve stimulus given before (-1 to -3 ms) or after TMS (+1 to +3 ms). **Sham STDP.** TMS coil will be positioned at Cz and peripheral nerve stimulation will be given to the tibial nerve as described above. **[D-cycloserine:** dose, potential toxicity, interactions with patients' medications. D-cycloserine is an FDA approved antibiotic for treatment of tuberculosis, urinary-tract infections, and management of schizophrenia and anxiety disorders (Otto et al., 2010; Kuriyama et al., 2011). A single 100 mg dose of D-cycloserine enhances motor learning in humans (Nitsche et al., 2004). D-cycloserine freely crosses the blood-brain-barrier, has modulatory activity, and can potentiate the excitatory response to NMDA, but because it has no excitatory activity on its own, the danger of excitotoxic damages, such that as seen with full NMDA agonists, is eliminated (Cascella et al., 1994). Dr. Perez lab has IRB approval to administer the drug in patients with SCI and controls.
Measurements will be taken before and after D-cycloserine or placebo (micro crystalline cellulose) administration (up to 6 placebo pills will be administered, a maximum of 3 for upper limb and a maximum of 3 for lower limb testing). To ensure efficacy of effects and safety we propose to give up to 8 (a maximum of 4 for upper limb and a maximum of 4 for lower limb testing, and up to) single doses of 100 mg orally of D-cycloserine 60-90 minutes before STDP to maximize its mechanisms of action.
The effects of NMDA receptor antagonist Dextromethorphan will be tested using the same procedures above. We will test the effect of an NMDA antagonist Dextromethorphan on blocking the plasticity to see if it is an NMDA mediated effect. A dose of 30 mg (or a placebo) orally will be administered as per safety regulations. We propose to study two novel strategies to strengthen corticospinal synaptic plasticity of upper and lower-limb muscles in individuals with SCI: a). administration of an NMDA antagonist (Dextromethorphan) with noninvasive paired stimulation, (Experiment 1) and b). Combine NMDA-induced plasticity with motor training (experiment 2). Spike timing-depending synaptic plasticity is thought to depend on activation of NMDA receptors (Dan and Poo 2004). Indeed, administration of NMDA receptor agonist enhances motor skill behaviors in both animals and humans (Nitsche et al., 2004; Grzeda and Wis´niewska 2008; Kuo et al., 2008; Kuriyama et al., 2011). A crucial strength of combining NMDA-induced-corticospinal synaptic plasticity with training is that it aims at enhancing motor training effects by promoting plasticity in the corticospinal pathway (Nitsche et al., 2004; Kuo et al., 2008). This approach is in agreement with the view that induced-plasticity combined with training can enhance the effectiveness of rehabilitation outcomes after SCI (García-Alías et al., 2009) and other motor disorders (Hummel and Cohen 2005). Our approach advances previous plasticity protocols by aiming to enhance not only corticospinal excitability but by strengthening synaptic connections onto spinal lower-limb motoneurons to promote recovery of leg clearance during locomotion. If the effects of medication + STDP are effective, we will combine medication + STDP + Training in upper and lower extremities.
Subjects will be asked to remain in the lab while the medications begin to take effect for 120-150 minutes.

When using the medications and placebos, both the subjects and the researcher will be blinded (double blind). The PI and the Study coordinator will know which medication was provided. Randomization of medication or placebo administration will be done by placing and selecting options "A" or "B" in an envelope corresponding to either the drug or the placebo.
Once all the data is collected and analyzed the researcher and study participant will be un-blinded.

The D-cycloserine, Dextromethorphan and the placebo (micro cellulose) pills used in this study will be prescribed by Dr. Howard Leven, M.D. of the Departments of Neurological Surgery and Orthopedics & Rehabilitation. The medications and placebo pills will be purchased from a licensed compound pharmacy and paid for by the grant. There is no monetary cost to the subjects for the medications.

Spinal Cord and Brain MRI:(to be conducted at any time during an individual's participation in this research; both SCI subjects as well as healthy controls will be asked to complete the MRI). We will explore the extent to which physiological and training outcomes correlate with the site and extent of the lesion defined by structural MRI of the spinal cord or brain. We will estimate the reduction in spinal cord width and area or volume (atrophy). Previous evidence showed a quantitative relationship between spinal cord atrophy and the extent of disability in individuals with SCI (15). We will examine the antero-posterior width (APW), left-right widths (LRWs) and cross-sectional spinal cord area (SCA). Since sensory pathways are predominantly located in the posterior spinal cord it is expected that atrophy in the APW will be correlated to sensory deficits, while atrophy in the LRW will be better correlated with motor deficits. Diffusion Tensor Imaging (DTI). DTI will be used to determine the pathophysiology of the spinal cord. We will measure the apparent diffusion coefficient and the fractional anisotropy (FA). Based on previous findings (16,17), we expect that the cord with lesion has a lower FA value than normal spinal cords. If the sensitivity is sufficient, we will reconstruct a 3-D representation of the spinal cord white matter tracts by using specific fiber-tracking algorithms. The MRI will also be used with Brainsight neuronavigation to more accurately pin point areas of TMS stimulation.

Measurements before and after STDP protocols:
A). TMS measurements. MEPs: TMS will be delivered to the optimal scalp position for activation of the dominant FDI and thumb muscles in controls and less affected side in patients. Measurements: resting (minimum intensity required to induce MEPs greater than 50 μV in 5/10 consecutive trials at rest), and active (minimum intensity able to evoke an MEP bigger than 200 μV in 5/10 consecutive trials during 10% of MVC) motor threshold. Thirty MEPs will be average in each condition. Cervicomedullary Motor Evoked Potentials (CMEPs): Supramaximal electrical stimulation will be administered posterior to the mastoid process to elicit motor evoked potentials to upper and lower limb muscles.

B). Motoneuronal excitability (reflected by F-wave persistence and amplitude): Using supra-maximum stimulus intensity (120% of the M-max; 0.2 ms duration, 30 trials) to the ulnar and median nerve at the wrist we will examine F-waves in FDI and thumb muscles. **C) EMG and force voluntary output:** Individuals will perform fast 10% of MVC into index finger abduction without corrections. Subjects will control a cursor on a computer screen up to 8% of MVC and the program will simulate the 2% of MVC needed to reach the target (Bunday and Perez, 2012). **D) Nine Hole Peg Test (9HPT):** This test measures finger dexterity. Nine small pegs are placed in a container and subjects are instructed to pick up each of the pegs and put them back as fast and accurate as possible. **E). Jebsen Taylor Test (JTT):** This test measures hand function. The time to complete subcomponents of the JTT will be measured. **F) Chedoke Arm and Hand inventory (CAHAI):** This test measures upper-limb motor recovery. Quantitative scale with 13 items (each item is given a score: 1 to 7).
H-Reflex of upper and lower extremities will be tested using DS7A electrical stimulator.
(G) Electrical Perceptual Threshold (EPT): This test provides a quantitative measure of sensory function by using small pulses of electricity on the surface of the skin and will be used to evaluate sensory threshold in the hands and arms.
Follow up assessments done at 1 and 3 months: We will evaluate the subject's ability to use their arms and hands using the different assessment tools listed above (A-G). They may be asked to complete a series of tasks such as stacking checkers, turning over cards, squeeze a device to determine how strong their grip is, and see how well you are able to feel sensations on the surface of their skin. TMS measurements, CMEPS and EMG recordings may also be done at these follow up assessments

Statistical and power analysis
Repeated-measures ANOVA and Tukey post hoc test will determine the effect of DRUG (excitatory STDP+D-cycloserine, inhibitory STDP+D-cycloserine, STDP+placebo), and TIME (0, 30 min, 1 hour, 3 hours and follow up 1 and 3 months) on MEPs, F-waves, force, and EMG. To obtain statistical power with

an alpha (type I error) of 0.05 and 1-beta (power) of 0.8, 28 people with SCI (14 in each group) and 28 age-matched healthy controls (14 in each group) will be included (Bunday and Perez, 2012).
The statistical and power analysis for Dextromethorphan will follow the same procedures as above.

Outcomes and interpretation
No results have been reported on the effects of STDP after SCI in combination with NMDA receptor agonist. It is expected that the presynaptic terminal has to be stimulated before the postsynaptic terminal to facilitate corticospinal and voluntary output. [Preliminary data indicate that STDP at 20 Hz for 20 min combined with D-cycloserine increases MEP size in a finger muscle in patients and in healthy controls. Notably, index finger force and FDI EMG activity increased in patients and controls to a larger extent that in our previous results (Bunday and Perez, 2012).] Therefore, it is expected that synaptic plasticity in the corticospinal pathway targeting finger muscles and leg muscles can be potentiated by changing activity in NMDA receptors activity, which will enhance voluntary output. [We anticipate similar results in thumb and other muscles since improvements in motor outputs were shown in healthy controls (Taylor and Martin, 2009).]

Pitfalls and Contingency
Lack of STDP effects. If our protocol does not elicit a plastic effect we will change stimulus intensity. [Since STDP was induced in hand and leg muscles after SCI we anticipate that similar parameters will be needed in our study. One muscle can be targeted at a time but due to the short duration of the protocol other muscles can be tested (i.e. wrist flexor and extensors, TA, ankle flexors and extensors) increasing the functional potential of our study by stimulating the proper nerve and cortical region.] STDP outcomes. STDP protocols outcomes will be expressed relative to their baseline value because different plasticity mechanisms might contribute to their effects.

EXPERIMENT 2. Approximately 100 sessions
In this experiment, patients and healthy controls will be randomly assigned to 2 groups: (1) training+STDP, and (2) training alone. Preliminary data showed that training+STDP facilitates voluntary output; thus, the expectation is that this protocol will be used prior training. Training will consist of 20 sessions in 4 wks. In each session, stimulation or sham stimulation (~20 min) will be applied at rest before training (~1 hr).

SCI and Controls - Upper Limb and lower Limb Testing
Group 1:

Locomotor Training + Sham STDP Facilitatory (20 sessions)
Locomotor training + STDP -Facilitatory protocol (20 sessions) (cortical and spinal stimulation)
Locomotor Training + D-Cycloserine+ STDP - Facilitatory protocol (20 sessions 1 session with D-cycloserine) (cortical and spinal stimulation)
Locomotor training + D-Cycloserine + Sham STDP (20 sessions 1 session with D- cycloserine) (cortical and spinal stimulation)

Group 2:
Training only -20 sessions

Upper limb training
Training will consist of grasping with the thumb and index finger targets of different diameters (1, 2.5, and 6 cm) in a vertical board (36x30") positioned in front of them. The board will contain 6 panels (12x15") which will light up individually by a computer cue. Each panel will have three LED lights to indicate grip strength to be exerted (red=30% MVC, blue=20% MVC, yellow=5% MVC). A reflective line in each target will identify the place to grasp, which will be modified to increase task difficulty. Force transducers will run along the sides of each target to communicate with LEDs. Kinematics and EMG signals will be acquired (EMG signals may be acquired through surface electrodes or intramuscular needles. For single muscle

fiber or motor unit potential recordings, a fine needle electrode will be inserted through the skin into the muscle. A ground electrode will be placed on skin of the arm. Intramuscular EMG will also be recorded during these contractions from different muscle sites to show the recruitment and firing rates of various motor units. Inter-spike intervals are used to calculate firing rates during small voluntary contractions. The force at which units are recruited is measured. Intramuscular EMG recordings are an optional measurement). Subjects will be instructed to grasp the object between index finger and thumb as fast and accurately as possible holding the final posture for 3 s until the light disappear. 300 trials will be completed. Training+sham: Training as above. For sham STDP see experiment 1.

Lower Limb Training

Locomotor training+ facilitatory STDP protocol: In each session, cortical or spinal STDP stimulation will be targeting the leg muscles at rest, and their effects will be tested on voluntary contractions. Locomotor training will be preceded by the STDP protocol. The locomotor training will consist of walking. The amount of weight support will be provided as needed by a Zero gravity system (ZeroG), and the level of support will be associated with gait kinematics which resemble unsupported walking. Zero Gravity system Robotic over ground body-weight support system for practicing a wide range of activities without the risk of falling..The support will be adjusted within and between sessions as necessary to prevent excessive knee flexion during stance phase or toe dragging during swing phase (Finch et al., 1991). Subjects will be encouraged to walk at their comfortable speed at which step kinematics are acceptable (no toe dragging, an adequate knee flexion during swing phase, adequate knee extension at initial stance phase, etc.). All subjects will be allowed to rest as needed during the training sessions. The starting and end points and guiding path will be marked with lines on the floor. Thirty-five retro-reflective markers will be attached to the participant's body according to the Plug-In-Gait market set. While the participant is walking on the path, joint movements will be acquired by a 3-dimensional motion capture system (VICON Motion Systems, Oxford, UK). The locomotor training session will consist of 20 sessions, and at least 3 sessions are expected to be completed per week as the participant is able. Missed sessions will be rescheduled according to the subject's availability. The assessments will be conducted before the first testing session and after the 20thsession. Also, assessments will be performed after every 5th sessions until completion. Possible facilitatory groups are: 1) locomotor training +cortical stimulation, 2) locomotor training +spinal stimulation, and 3) locomotor training +cortical and spinal stimulations. Subjects may partake in multiple groups. Participants wanting to partake in multiple groups will have a rest period of at least 1 week between groups.

Locomotor Training + D-cycloserine + facilitatory STDP Protocol: The STDP stimulation will be applied after taking a single dose of 100 mg of D-Cycloserine. The methods followed thereafter are the same as above. **Locomotor training + D-cycloserine + Sham STDP protocol**: In control session, repetitive cortical and spinal stimulation (TMS) will be used to compare the main effects. Training will be conducted as described above but a sham cortical or spinal STDP will be applied. Corresponding control group will be considered as needed. Possible control groups are: 1) locomotor training+cortical sham stimulation, 2) locomotor training+spinal sham stimulation, and 3) locomotor training+cortical and spinal sham stimulations.

Measurement before and after Locomotor training+D-cycloserine+facilitatory (STDP) and Locomotor training+D-cycloserine+control (sham STDP) protocol: We will pair presynaptic volleys (produced by TMS) and postsynaptic volleys (produced by PNS) to arrive at the spinal cord. 100 pairs of stimuli will be delivered at 0.1 Hz. TMS intensity will be adjusted to produce an MEP of 50% of MEP-max on the muscle tested. PNS intensity will be set to evoke an M-max. The ISI will be estimated from the latencies of MEPs, M-max, and F-waves. The time of impulses arriving at the presynaptic terminal will be based on the central conduction time calculated as: MEP latency - (F-wave latency + M-max latency - 1/2). The time of impulses arriving at the postsynaptic terminal will be based on the peripheral conduction time calculated as: F-wave latency + M-max latency - 1/2. The ISI between paired pulses will be timed to allow descending volleys to arrive at the presynaptic terminal of corticospinal neurons 1-2 ms before antidromic volleys in motoneurons reached the dendrites. Based on our preliminary data and previous results, in the facilitatory protocol action potentials arrived at the presynaptic terminal around 2 ms before motoneuronal discharge. These are the times required to elicit spike timing-dependent plasticity. **Sham STDP protocol:** We will use the same stimulus intensities described above for TMS and PNS. Here, the ISI between paired pulses will be timed to allow antidromic volleys to reach motoneuron dendrites 5 ms before the descending

volleys reached the presynaptic terminal.. Here, the TMS coil will be positioned at the area for better activation for appropriate muscles and peripheral nerve stimulation will be given to the corresponding nerve at intensities described above at an ISI the allows pulses to arrive at the presynaptic terminal around 2 ms before motoneuronal discharge. Our preliminary data shows that the same interval is required for QUAD and HAMS because of latency similarities in peripheral and cortically evoked responses. We found that similar amount of plasticity can be elicited by targeting QUAD when QUAD-HAMS are targeted simultaneously. This might open the possibility during training to target three instead of two muscle representations.

Measurements before and after Paired Stimulation Protocols: Measurements will be taken before and immediately after and up to 60 min after stimulation. Furthermore, basic stride characteristics will be obtained based on the captured 3-dimensional joint movements including walking speed [m/s], cadence [steps/min], stance phase percentage in gait cycle [%], stride time [s] stride length [m]. In addition, the participant's kinematics will be analyzed; joint angles of shoulder, hip, knee, and ankle will be calculated to analyze the shoulder, hip, and knee flexion/extension and ankle plantar/dorsiflexion (Davis et al., 1991).

Statistical and power analysis: Repeated-measures ANOVA and Tukey post hoc test will be used to determine the effect of TRAINING on MEPs, F-waves, and kinematics. To obtain a statistical power with an alpha (type I error) of 0.05 and 1-beta (power) of 0.8, 15 individuals on each group will be considered (Kuo et al., 2008).

Outcomes and interpretation of training protocols: Administration of NMDA receptor antagonists can prohibit long-term synaptic potentiation, thus preventing skill-learning practice from translating into successful skill acquisition in animals (White et al., 1998). On the other hand, the administration of NMDA receptor agonists enhances skill-learning in animals (Grzeda and Wis´niewska 2008) and humans (Kuriyama et al., 2011). NMDA receptor-dependent glutamatergic neurotransmission contributes to the formation of long-term memories (Herron et al., 1996; Barker and Warburton 2008). Thus, it is likely that D-Cycloserine will enhance training induced improvements via enhancing NMDA receptor-dependent synaptic plasticity. Our preliminary data show that individuals with SCI and healthy controls can learn to control a cursor by using EMG signals from upper-limb muscles. After one training session, the overall error decreased in individuals with SCI. To understand the source of the decreased error we examined EMG signals in relation to the target path and to each other. It is expected that improvements in performance will involve a linear increase in lower-limb muscle EMG activity. It is expected that after repeated training subjects will learn to balance lower-limb muscle activity to accomplish the goal. It is less likely that these results are related to the use of only one muscle since we examined individual muscles and the target path. This approach might allow future interventions to target specific muscles which are more affected by the injury.

Pitfalls and Contingency: *MVC levels.* We will test EMG levels up to 30% of MVC. The assumption of linearity is present at this low range but at higher MVC levels this might not be adequate. However, since most of our daily activities involve less than 30% of MVC these levels are close to mimic daily functions. *Muscle weakness.* % of MVC use during training will be normalized to each individual. Since EMG and force demonstrated similar linear improvements (shown by preliminary data) both signals can be used for training. *Training plateau.* To avoid plateau and maximize learning we will include linear and non-linear target paths at different force levels within the 0-30% range. *Number of muscles.* We will test 2 muscles while most movements involve several muscle groups. In principle, this approach should also work with a larger number of muscles. Considering that controlling a cursor was difficult for people with SCI we choose a simpler design which can be potentially expanded for future studies.

Measurements before and after training

1). Kinematics: measured during self-paced and fast grasping or walking movements recorded by six Natural Point OptiTrack V100 cameras (120 Hz) and by Opti Track, V120: Trio camera, NaturalPoint, Inc, with10 cameras respectively for the locomotor training. Subjects will be seated and grasp with the less affected (patients) and dominant (controls) arm. Cameras will be positioned in front/sides of subjects with

markers on the tip of the thumb and index finger, inner side of the wrist, lateral size of the elbow and shoulder joints. Variables: 1) Movement
onset (MO): time between the GO signal and start of forward arm velocity. 2) Total movement duration (MD): time between MO and grasp (Gr=time when index and thumb contact target). MD will be broken in time to reach maximum velocity (MV), maximum hand aperture (MA), and the first finger contact (FC), and Gr.
2). Hand and leg trajectory: individual trials will be normalized to the initial trajectory defined as a line from the initial to the end position. Trajectory data will be rotated to be aligned and scaled to compare among trials and subjects. 3). Grasping accuracy: distance between thumb and index finger and place to grasp. 4).Physiological/behavioral tests (see experiment 1).
Follow up assessments done at 1, 2 and 3 months: We will evaluate the subject's ability to move their legs, feet or toes using the different physiological and behavioral tests listed in experiment 1. TMS measurements, CMEPS and EMG recordings may also be done at these follow up assessments

Statistical and power analysis
Repeated-measures ANOVA and Tukey post hoc analysis will determine effects of GROUP (training+STDP, training+sham), TIME (sessions 1, 5, 10, 15 and follow-up 1, 2 and 3 months) on physiological and behavioral outcomes. For alpha (type I error) of 0.05 and 1-beta (power) of 0.8, 30 SCI patients (15/group) and 30 healthy controls (15/group) will be included (Bunday and Perez, 2012).

Pitfalls and Contingency
Grasping individual differences. Because variations in the starting position, arm length, and reach can affect the total movement distance, we will scale data to each individual maximum reach. Since subjects can reach at different speeds, and from trial to trial, we will resample the trajectory data into 0.1% equidistant time points. Training plateau. To avoid learning plateau we will increase task difficulty by changing target orientation (0-90º) and force (0-30% MVC).

Target population:

Male and female veterans and non-veterans with spinal cord injury at least 6 months after injury (≥ 2 months of injury for EPT and ASIA) was sustained. We also plan to enroll control subjects who do not have any history of spinal cord injury.

Inclusion Criteria:
Participants who are unimpaired healthy controls:
(1) Male and females between ages 18-85 years
(2) Right handed
(3) Able to complete precision grips with both hands
(4) Able to complete full wrist flexion-extension bilaterally
(5) Able to walk unassisted
(6) Able to complete full ankle flexion-extension bilaterally

Participants who have had a spinal cord injury:
(1) Male and females between ages 18-85 years
(2) SCI at least 6 months post injury for TMS (≥ 2 months of injury for EPT and ASIA)
(3) Spinal Cord injury at or above L5
(4) The ability to produce a visible precision grip force with one hand
(5) Able to perform some small wrist flexion and extension
(6) The ability to perform a small visible contraction with dorsiflexion and hip flexor muscles
(7) No subjects will be excluded based on their race, religion, ethnicity, gender or HIV status.
(8) ASIA A,B,C, or D

Exclusion criteria for enrollment For SCI and Healthy Control Subjects (4-8 exclusion for non-invasive brain stimulation only):

(1) Uncontrolled medical problems including pulmonary, cardiovascular or orthopedic disease
(2) Any debilitating disease prior to the SCI that caused exercise intolerance
(3) Premorbid, ongoing major depression or psychosis, altered cognitive status
(4) History of head injury or stroke
(5) Metal plate in skull
(6) History of seizures
(7) Receiving drugs acting primarily on the central nervous system, which lower the seizure threshold (see appendix 2)
(8) Pregnant females
(9) Ongoing cord compression or a syrinx in the spinal cord or who suffer from a spinal cord disease such as spinal stenosis, spina bifida, MS, or herniated disk
(10) Individuals with scalp shrapnel, cochlear implants, or aneurysm clips.

The following exclusion criteria from above apply only to TMS:
Numbers 3-10

Qualified subjects can participate in up to 275 visits for this study. Visit range from 1-4 hours in length.

Start date: 11/2015.
Completion Date: 12/2019

D. N/A No procedures are being performed for diagnostic purposes.

E. Risks

This is a minimal risk proposal.

TMS procedures: The TMS system stimulates the brain non-invasively. It generates a small magnetic field across the subject. TMS is widely used in clinical research, and the risks of TMS are believed to be very low. However, there is the very slight chance that TMS can cause a seizure. There is no ionizing radiation exposure involved and the studies are non-invasive. All participants will be screened before enrollment to assure that they meet study criteria and that there are no contraindications to TMS.

Subjects will be instructed that they can discontinue the TMS experiment at any time. In the unlikely event of a seizure, a medical physician will be contacted for immediate response. If a headache or mild scalp discomfort occurs, subjects will be directed to use over the counter pain medication at their own discretion. Earplugs will be utilized to minimize any type of hearing damage that might possibly occur due to the sound generated by the TMS equipment. Dr. Perez (Co-PI) will be present during all TMS procedures.

Dr. Perez has been working with TMS procedures for the last 15 years and has extensive experience in the use of TMS procedures. Although very unlikely, it is theoretically possible that the participant may have a seizure induced by the TMS. Considering the large number of subjects and patients who have undergone TMS studies since 1998 and the small number of seizures, we can assert that the risk of TMS to induce seizures is certainly very low (see review by Rossi et al., 2009). However, we will inform the participant about this unlikely risk. If a seizure occurs, it will occur during the TMS application itself, not after. When a seizure happens the participant's brain starts acting strangely and participants may feel dizzy and have repetitive rhythmic movements of any part of their body. If this unlikely event happens, we will immediately call an attending physician. On weekends, the subject should call the emergency 24 hour pager 305-243-1000 to get in touch with Dr. Levene , and let him know that he is a research subject from the Miami VA in Maximizing spike timing dependent plasticity after Spinal Cord Injury.

In the unlikely event of a seizure, a standard seizure protocol will be followed:

1. Cushion the head and area so subject does not injure themselves.
2. Turn the subject on their side to prevent aspiration

3. Monitor the duration of the seizure
4. If the seizure ends in less than 60 seconds, monitor and contact the primary care MD to rule our predisposing factors.
5. If the seizure lasts more than 60 seconds, activate emergency services.

In the event that a seizure occurs on premises and we are unable to reach the physician, 911 will be called for emergency services.

D-Cycloserine: Common risks include headache, confusion, drowsiness, somnolence. Infrequent risks include severe allergic reactions (rash; hives; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); hepatotoxicity; Megaloblastic Anemia; congestive heart failure; visual disturbances. Other risks include Neurological side effects may occur with long-term use of higher doses (> 500mg daily). These may include: Convulsions, Tremor, Dysarthria, Vertigo, Psychoses, possibly with suicidal tendencies, Character changes, Hyperirritability, Aggression, Paresis, Hyperreflexia, Paresthesia, Major and minor (localized) clonic seizures, Coma. Contraindications to the use of D-cycloserine include epilepsy, depression, anxiety disorder, psychiatric disorder, kidney disease, or alcohol abuse. Risks that are listed for the taking of D-cycloserine and Dextromethorphan have been supplied. These risks and their frequency have been documented in target patient populations. Subjects who report any of the following will be excluded from participation in additional visits using D-cycloserine: epilepsy, depression, anxiety disorder, psychiatric disorder, kidney disease, or alcohol abuse. Additionally, subjects who report using an MAO inhibitor in the past 14 days will be excluded from participation in Dextromethorphan visits. As this is a one-time dose in a controlled environment, the likelihood of these risks occurring is minimal. Also, subjects will remain in the offices of the PI's research lab until it is deemed that it is safe for the subject to return home.

Dextromethorphan: Common risks include Nausea, Stomach pain, Drowsiness and Dizziness. Infrequent risks include severe allergic reactions (rash; vomiting; shallow respiration; hypertension); Ataxia; Toxic psychosis (hyperactivity, marked visual and auditory hallucinations); Coma. Other risks include Insomnia, Dysphoria (anxiety, nervousness) and Depression may occur with long-term use. Risks that are listed for the taking of D-cycloserine and Dextromethorphan have been supplied. These risks and their frequency have been documented in target patient populations. Subjects who report any of the following will be excluded from participation in additional visits using D-cycloserine: epilepsy, depression anxiety disorder, psychiatric disorder, kidney disease, or alcohol abuse. Additionally, subjects who report using an MAO inhibitor in the past 14 days will be excluded from participation in Dextromethorphan visits. As this is a one-time dose in a controlled environment, the likelihood of these risks occurring is minimal. Also, subjects will remain in the offices of the PI's research lab until it is deemed that it is safe for the subject to return home.

Data and safety monitoring: Vital signs including blood pressure, heart rate, and respiratory rate will be monitored before and after each of the sessions involving mediations. We will also be monitoring for the side effects of D-Cycloserine and dextromethorphan as listed in the protocol. After a few subjects (2-3) have completed that phase of testing, the PI will meet with study team members and the prescribing physician to evaluate the study data and safety concerns. Other aspects of the study and/or safety concerns will be discussed by the PI and study team members at least monthly in scheduled study team meetings.

Electromyography(EMG) Surface EMG recording: Experimental interventions: Local discomfort or itching at the site of the recording electrode. Intramuscular EMG recording
The primary risks when using needle electrodes are pain, bleeding and infection. The risk of infection will be minimized by using sterilized needles.

Risks of Muscle Activity Recording (EMG): Mild discomfort, such as an itching sensation may be felt under the electrodes that register the response in your muscles. There may be some pain or bleeding from the needles used for intramuscular recording. The possibility of this is infrequent (occurring in 1-

10%, or 1-10 out of 100 people). We will clean the area with alcohol and use standard, hospital grade electrodes, and sterile needles.

Peripheral Nerve Stimulation intensity will be increased gradually during the evaluations when necessary. Mild discomfort, such as an itching sensation may be felt under the electrode that stimulates your wrist or under the electrodes that register the response in your muscles. The possibility of this is infrequent (occurring in 1-10%, or 1-10 out of 100 people). We will clean the area with alcohol and use standard, hospital grade electrodes. Another risk of the nerve stimulation used in this research is that you might feel mild pain, if pain fibers could be activated.

MRI scan: Subjects, investigators, and facility staff will be examined by a hand-held screening device for magnetic material before entry and each re-entry to the scan room. Warning signs, including the specific dangers of high magnetic fields, are posted in the necessary location. The standard 3 Tesla Magnet will be used for testing. The MRI exam involves no exposure to x-rays or radioactivity, and is safe. The FDA approved the Siemens 3-T scanner, which time varying magnetic fields (gradient), specific absorption rate and acoustic noise levels. FDA guidelines will be strictly enforced at our 3T scanner.

JTT/CAHA/pinch grip/ASIA/EPT: individuals will have resting periods as needed, all procedures will be explained in detail and subjects will be informed that they can stop the experiment at any time

Screening Interview: Identifiable information collected during either the screening interview will be destroyed if the potential subject does not qualify. If the subject does qualify and ultimately enrolls into this research, all information collected will become part of their research record. All security precautions that are reflected in this protocol will apply.

Data Collection and Storage: Research records will be retained in accordance with the Veterans Health Administration (VHA) Records Control Schedule. The Federal Privacy Act protects the confidentiality of medical records. Electronically-stored medical information collected from study participants will be kept on a VA server or on a UM secure server. Electronic data recorded during experimental sessions will be kept on fully-secured computers (password protected) on a VA server or on a UM server. Unique subject identifiers (i.e., a code accessible only to the PI and AI) will be used to label all electronic data files. The transferring of any data will be done by using a VA secure flash drive. Hard copies of data will be stored at the Miami VA in room 1C103 in a locked cabinet.

F. Sample Size

Up to 500 participants (250 SCI, and 250 control subjects Veterans and non-veterans) will be recruited to participate in this study at the VAMHS and at the University of Miami. Based on our previous protocols testing spinal cord injured subjects and healthy control subjects, we have added a 25% attrition rate to the number of subjects we plan to enroll in the study.

G. Data analysis is consistent with the study objective

Aim 1 Experiment 1. Repeated-measures ANOVA and Tukey post hoc test will determine the effect of DRUG (excitatory STDP+D-cycloserine, inhibitory STDP+D-cycloserine, STDP+placebo), and TIME (0, 30 min, 1 hour, 3 hours and follow up 1 and 3 months) on MEPs, F-waves, force, and EMG. To obtain statistical power with an alpha (type I error) of 0.05 and 1-beta (power) of 0.8, 28 people with SCI (14 in each group) and 28 age-matched healthy controls (14 in each group) will be included (Bunday and Perez, 2012).

Aim 1 Experiment 2: Repeated-measures ANOVA and Tukey post hoc analysis will determine effects of GROUP (training+STDP, training+sham), TIME (sessions 1, 5, 10, 15 and follow-up 1, 2 and 3 months) on physiological and behavioral outcomes. For alpha (type I error) of 0.05 and 1-beta (power) of 0.8, 30 SCI patients (15/group) and 30 healthy controls (15/group) will be included (Bunday and Perez, 2012).

H. Privacy and Confidentiality

Every effort will be made to make sure that the information about you obtained from this study will be kept strictly confidential. Private information is collected about participants during the screening sessions as part of this study. Once the necessary information is collected it is placed into a secure cabinet in a locked room. The research staff will take every precaution to protect all identity and the confidentiality of the information collected about each subject. Any electronic or hard/paper copies of the information collected will be stored in a secured location. Any copies that contain information that could be used to identify participants (such as their name, address, date of birth, etc.), will be stored separately from any information that does not contain identifiers. Only those individuals who are authorized to review the information will have access to it. Identifiable electronic information related to participants' involvement will be stored on restricted access password protected servers. In order to protect their confidentiality, data that we record about subjects may be sent to the organizations listed above via a secure website, courier and or facsimile. The data that will be shared with the study sponsor will not include any names or any information that may directly identify participants. All data will be coded with the study number, which may include participants' initials.

Information may also be disclosed to the VA Miami Healthcare System Research and Development Office Staff in order to perform duties related to research administration.

Recruitment Methods

Subjects will be recruited through research registries at the Miami VA and the University of Miami and The Miami Project to Cure Paralysis. Advertisements for the studies will be posted around the UM campuses, and the VA Miami Campus. Interested parties will call or email. A screening session will be scheduled for SCI participants. For control subject there will be a screening form that will be filled out either in person or over the phone. Screening procedures will take place at the VA Miami room 1C103 or at the University of Miami, in the Miami Project to cure paralysis in Dr. Monica Perez's Lab rooms 140-156. Informed consent forms will be provided and explained to all participants prior to any in person screening session. Informed consent for SCI participants will be done at the VA or at UM, and for controls at UM only.

Drugs The medications (dextromethorphan and d-cycloserine) listed in this protocol will be obtained through a prescription by one of the physicians on the protocol (Dr. Levene) and
will be purchased from either the UM compound pharmacy or another licensed pharmacy depending on price and availability.
The medications will be stored in room 1-56 at the Miami Project to Cure paralysis in a locked cabinet. Administration and dispensation of the all medications will be kept in a log book in Dr. Monica Perez's Lab, room 1-56. The log book will include the name of the participant receiving the medication, the date, which medication, the name of the research personnel dispensing the medication, and the signature of the PI.

Appendix 2

Medications that may lower seizure threshold

Intake of one or a combination of the following drugs forms a strong potential hazard for application of rTMS due to their significant seizure threshold lowering potential: imipramine,

amitriptyline, doxepine, nortriptyline, maprotiline, chlorpromazine, clozapine, foscarnet, ganciclovir, ritonavir, amphetamines, cocaine, (MDMA, ecstasy), phencyclidine (PCP, angel's dust), ketamine, gamma-hydroxybutyrate (GHB), alcohol, theophylline.

In these cases rTMS should be performed, when required, with particular caution.